

Interferon- γ affects leukemia cell apoptosis through regulating Fas/FasL signaling pathway

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Abstract. – **OBJECTIVE:** Imbalance of hematopoietic cell proliferation and apoptosis is one of the major causes of leukemia. Enhanced cell proliferation and reduced apoptosis lead to hemocytes accumulation. Fas/FasL signaling pathway promotes cell apoptosis. This study investigated the impact of interferon γ (IFN- γ) on chronic myelogenous leukemia cell proliferation and apoptosis to elucidate its interaction with Fas/FasL signaling pathway.

PATIENTS AND METHODS: Leukemia K562 cells were routinely cultivated and treated with 10 U/ml, 100 U/ml, and 1000 U/ml interferon for 12 h, 24 h, and 48 h, respectively. MTT assay was applied to test cell proliferation. TUNEL assay was adopted to determine cell apoptosis. Western blot was selected to detect Fas/FasL expression.

RESULTS: Different concentrations of IFN- γ inhibited cell proliferation at various time points. IFN- γ at 1000 U/ml treatment for 48 h exhibited the strongest suppressive effect on cell proliferation ($p < 0.05$). IFN- γ intervention enhanced K562 cell apoptosis with concentration and time dependence ($p < 0.05$). Fas and FasL proteins expressions upregulated after treated by IFN- γ following dose elevation and time extension ($p < 0.05$).

CONCLUSIONS: IFN- γ inhibits leukemia K562 cell proliferation and promotes cell apoptosis via facilitating Fas and FasL proteins expressions.

Key Words:

IFN- γ , Leukemia, Fas/FasL.

Introduction

The pathogenesis of leukemia is mainly caused by leukemia cells uncontrollable malignant proliferation from the bone marrow and other tissues. Leukemia may appear when they enter the peripheral blood¹. Following the increase of

inflammatory cells, leukemia keeps on progression and infiltration, suggesting that leukemia cells can escape the immune surveillance². It is found that Fas/FasL signaling pathway plays an important role in cell apoptosis and immune surveillance³. Fas/FasL signaling pathways participate in inducing cell apoptosis, which is an important component of the metabolic process that can effectively remove aged cells. Moreover, it can timely remove the abnormal cells and trigger a series of immune reactions^{4,5}. Interferon- γ (IFN- γ) involves in cell apoptosis process and can regulate the expression of apoptosis-related genes⁶. In chronic myelogenous leukemia, IFN- α upregulates dendritic cell costimulatory molecules and MHC antigen molecules expressions, thus has a certain stimulatory effect on T cell immune response⁷. However, the mechanism of IFN- γ on chronic myelogenous leukemia is still unclear. This study selected leukemia K562 cells and applied IFN- α for intervention, aiming to investigate the IFN- γ on chronic myelogenous leukemia cell proliferation and apoptosis, and elucidate its interaction with Fas/FasL signaling pathway. MTT assay was applied to test cell proliferation. TUNEL assay was adopted to determine cell apoptosis. Western blot was selected to detect Fas/FasL expression.

Patients and Methods

Experimental Cells

Leukemia K562 cells were provided by the Shanghai Institute of Hematology, China.

Reagents

IFN- γ was purchased from Sinopharm Chemical Reagent Co., Ltd (Lot No. 20050913) (Bei-

jing, China). TUNEL assay kit, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT), rabbit anti-mouse Fas and FasL polyclonal antibodies, and goat anti-rabbit secondary antibody were provided by Santa Cruz Biotechnology (Santa Cruz, CA, USA). Dulbecco's Modified Eagle Medium (DMEM) medium, penicillin-streptomycin, and fetal bovine serum were got from Gibco (Thermo Fisher Scientific, Waltham, MA, USA).

Routine Cell Culture

K562 cells were cultured in Roswell Park Memorial Institute-1640 (RPMI-1640) medium at 37°C and 5% CO₂.

IFN- γ Intervention

Cells in logarithmic phase were incubated in 2% FBS (fetal bovine serum) for 24 h and further cultured in DMEM containing 10% FBS. IFN- γ was added to treat the cells, while untreated K562 cells were selected as control.

MTT Assay

Different concentrations of IFN- γ (10 U/ml, 100 U/ml, and 1000 U/ml) were used to treat cells for 12 h, 24 h, and 48 h. MTT at 5 mg/ml was adopted to incubate the cells for 4 h. The reaction was stopped by 150 μ l DMSO (Dimethyl sulfoxide) and the plate was tested at 570 nm.

TUNEL Assay

The cells were treated with dimethylbenzene, gradient ethanol, and Proteinase K in sequence. Next, the cells were incubated with TUNEL mixture, converter-POD, and DAB. At last, the cells were counted after redyeing.

Western Blot

The cells in logarithmic phase were treated by IFN- γ . A total of 40 μ g protein was separated by electrophoresis and incubated in primary antibody (1:200, β -actin 1:500) for 30 min. Next, the

membrane was incubated in secondary antibody (1:2000) for 1 h and developed. The membrane was scanned and analyzed by Quantity One software.

Statistical Analysis

SPSS 17.0 software (SPSS Inc., Chicago, IL, USA) was selected for data analysis. Enumeration data was tested by chi-square test, while measurement data was depicted as mean \pm standard deviation and compared by ANOVA followed by Turkey's multiple comparison tests. $p < 0.05$ was considered statistically significant.

Results

MTT Assay Detection of IFN- γ Impact on K562 Cell Proliferation

Different concentrations of IFN- γ were used to treat K562 cells for 12 h, 24 h, and 48 h. Different concentrations of IFN- γ inhibited cell proliferation at various time points. IFN- γ at 1000 U/ml treatment for 48 h exhibited the strongest suppressive effect on cell proliferation ($p < 0.05$) (Table I).

TUNEL Assay Detection of IFN- γ Intervention on 562 Cell Apoptosis

TUNEL assay was used to detect K562 cell apoptosis treated by IFN- γ . IFN- γ intervention enhanced K562 cell apoptosis with concentration and time dependence ($p < 0.05$) (Table II, Figure 1).

Fas and FasL Proteins Expressions in K562 Cells Treated by IFN- γ

Western blot was selected to detect Fas and FasL proteins expressions in K562 cells treated by IFN- γ for 12 h, 24 h, and 48 h. Fas and FasL proteins expressions upregulated after treated by IFN- γ following dose elevation and time extension ($p < 0.05$) (Table III, Figure 2).

Table I. IFN- γ affected K562 cell proliferation ($x \pm s$, %).

Time	Experimental group			Control
	10 U/mL	100 U/mL	1000 U/mL	
12h	0.985 \pm 0.072 ¹	0.723 \pm 0.046 ^{1,2}	0.232 \pm 0.024 ^{1,2,3}	0.091 \pm 0.023
24h	0.811 \pm 0.045 ^{1,4}	0.512 \pm 0.034 ^{1,2,4}	0.176 \pm 0.016 ^{1,2,3,4}	0.072 \pm 0.012
48h	0.321 \pm 0.031 ^{1,4,5}	0.256 \pm 0.026 ^{1,2,4,5}	0.121 \pm 0.011 ^{1,2,3,4,5}	0.034 \pm 0.016

¹ $p < 0.05$, compared with control. ² $p < 0.05$, compared with 10 U/ml. ³ $p < 0.05$, compared with 100 U/ml. ⁴ $p < 0.05$, compared with 12 h. ⁵ $p < 0.05$, compared with 24 h.

Table II. IFN- γ affected K562 cell apoptosis ($x \pm s$, %).

Time	Experimental group			Control
	10 U/mL	100 U/mL	1000 U/mL	
12h	11.18 \pm 1.02 ¹	17.27 \pm 1.44 ^{1,2}	21.24 \pm 2.45 ^{1,2,3}	3.01 \pm 1.26
24h	15.21 \pm 1.33 ^{1,4}	18.48 \pm 1.67 ^{1,2,4}	24.37 \pm 2.63 ^{1,2,3,4}	3.21 \pm 1.43
48h	18.26 \pm 1.54 ^{1,4,5}	20.85 \pm 2.12 ^{1,2,4,5}	28.92 \pm 2.77 ^{1,2,3,4,5}	3.32 \pm 1.39

¹ $p < 0.05$, compared with control. ² $p < 0.05$, compared with 10 U/ml. ³ $p < 0.05$, compared with 100 U/ml. ⁴ $p < 0.05$, compared with 12 h. ⁵ $p < 0.05$, compared with 24 h.

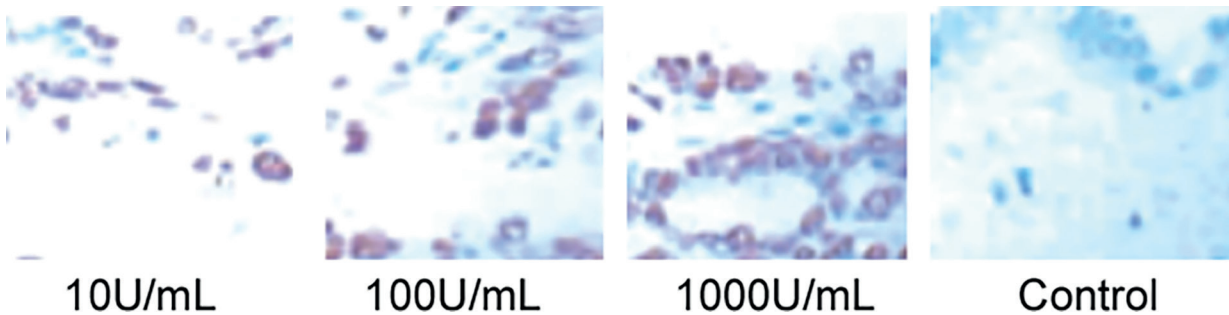


Figure 1. IFN- γ affected K562 cell apoptosis.

Discussion

Apoptosis can remove the redundant, poorly differentiated, and aged cells that are difficult to adapt to the state, thus to maintain the normal physiological function⁸. Imbalance of the hematopoietic cells proliferation and apoptosis may cause the occurrence of leukemia⁹. Fas/FasL signaling pathway mediated apoptosis plays an important role in tumor occurrence and development. It was pointed out that Fas did not show significant changes in most benign tumors compared with normal tissue. However, it was

reported¹⁰ that Fas downregulated in malignant tumors. It means that malignant tumor progress may be related to Fas downregulation or deletion, leading to Fas/FasL signaling pathway dysfunction. Malignant tumor cells may evade immune attack and reduce sensitivity to T lymphocytes by decreasing Fas expression on cell surface^{11,12}. IFN- γ can suppress malignant tumor cell proliferation, elevate MHC antigen expression, and inhibit angiogenesis¹³. This study adopted IFN- γ to treat leukemia K562 cells to analyze its function on chronic granulocytic leukemia cell proliferation and apoptosis.

Table III. Fas and FasL proteins expressions in in K562 cells treated by IFN- γ ($x \pm s$, $\mu\text{mol/l}$).

Item	Experimental group			Control
	10 U/mL	100 U/mL	1000 U/mL	
Fas				
12h	0.11 \pm 0.21 ¹	0.26 \pm 0.18 ^{1,2}	0.33 \pm 0.11 ^{1,2,3}	0.74 \pm 0.37
24h	0.34 \pm 0.15 ^{1,4}	0.44 \pm 0.13 ^{1,2,4}	0.41 \pm 0.03 ^{1,2,3,4}	0.72 \pm 0.38
48h	0.43 \pm 0.13 ^{1,5}	0.57 \pm 0.09 ^{1,2,5}	0.64 \pm 0.01 ^{1,2,3,4,5}	0.69 \pm 0.41
FasL				
12h	0.13 \pm 0.21 ¹	0.27 \pm 0.18 ^{1,2}	0.39 \pm 0.11 ^{1,2,3}	0.10 \pm 0.09
24h	0.34 \pm 0.25 ^{1,4}	0.42 \pm 0.21 ^{1,2,4,5}	0.54 \pm 0.12 ^{1,2,3,4}	0.10 \pm 0.08
48h	0.46 \pm 0.39 ^{1,4,5}	0.59 \pm 0.24 ^{1,2,4,5}	0.73 \pm 0.17 ^{1,2,3,4,5}	0.11 \pm 0.07

¹ $p < 0.05$, compared with control. ² $p < 0.05$, compared with 10 U/ml. ³ $p < 0.05$, compared with 100 U/ml. ⁴ $p < 0.05$, compared with 12 h. ⁵ $p < 0.05$, compared with 24 h.

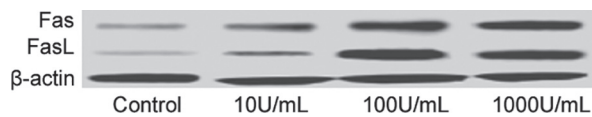


Figure 2. Fas and FasL proteins expressions in K562 cells treated by IFN- γ .

In this investigation, we used different concentrations of IFN- γ to treat K562 cells for 12 h, 24 h, and 48 h. It was found that different concentrations of IFN- γ inhibited cell proliferation at various time points. IFN- γ at 1000 U/ml treatment for 48 h exhibited the strongest suppressive effect on cell proliferation, indicating that IFN- γ can suppress K562 cell proliferation with dose-time dependence. It was reported¹⁴ that IFN- γ increased CML-DCs costimulatory molecules and MHC antigen molecules expressions, and promoted T cell proliferation. IFN- γ can elevate the number of DC with normal function and has a certain corrective effect on DCs with function defect¹⁵.

This study detected K562 cell apoptosis affected by IFN- γ . It was showed that IFN- γ intervention enhanced K562 cell apoptosis with concentration and time dependence, revealing that IFN- γ can promote leukemia K562 cell apoptosis. It was confirmed that Fas-mediated cell apoptosis was inhibited in malignant cells. Elevating Fas expression to enhance cell sensitivity to Fas-mediated cell apoptosis is an important mechanism of IFN- γ to trigger apoptosis. Researches¹⁶ demonstrated that IFN- γ can trigger Fas expression and increase cell apoptosis.

This study further adopted Western blot to test Fas and FasL expressions in K562 cells treated by IFN- γ . Fas and FasL proteins expressions upregulated after treated by IFN- γ following dose elevation and time extension, indicating that IFN- γ can upregulate Fas and FasL levels in K562 cells. A previous study¹⁷ suggested that IFN- γ can enhance Fas expression in cholangiocarcinoma and gastric cancer with dose-time dependence. Nevertheless, IFN- γ can elevate FasL expression in various malignant tumors. Fas is a type of receptor, while FasL is a legend. Their binding may induce immune system attack, leading to cell apoptosis¹⁸. Under the function of IFN- γ , Fas/FasL signaling pathway can induce malignant tumor cell apoptosis. FasL can bind with Fas to cause malignant tumor cell apoptosis^{19,20}. It was proposed that IFN- γ induction may enhance the apoptosis of leukemia cells treated with cytarabine chemotherapy. For the chemotherapy

drug targeting Fas/FasL, application of IFN- γ can produce a synergistic effect to enhance the therapeutic effect.

Conclusions

IFN- γ can suppress K562 cell proliferation and induce cell apoptosis by elevating Fas and FasL proteins expressions. Fas/FasL signaling pathway mediated cell apoptosis involves multiple cytokines with complicated mechanism. Further in-depth investigation is still needed in the pathogenesis, chemotherapy resistance, and immune surveillance of leukemia. A malignant tumor may be classified upon Fas/FasL level in the future to further analyze the prognosis. Fas/FasL signaling pathway provides new thinking and strategy for the treatment of leukemia, which is of great significance for the early diagnosis and clinical treatment of leukemia.

Conflict of Interest

The Authors declare that they have no conflict of interests.

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